Reproductive Biology of Male Brown Treesnakes (*Boiga irregularis*) on Guam

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Abstract.—Reproductive biology of males in the Guam population of the Brown Treesnake, *Boiga irregularis*, was investigated through monthly examinations of the urogenital system organs and plasma testosterone levels. All males examined during the 12 consecutive months of the study were spermatogenic and had sperm in the ductus epididymis and ductus deferens. No evidence of testicular recrudescence or regression was observed. Testis mass did not vary among months. Epithelial height of the kidney sexual segment was the only feature examined that varied significantly among months, with lowest heights observed in May through July. Despite this variation, the sexual segment in all males was hypertrophied and contained secretory granules. Plasma testosterone levels did not vary significantly among months, were relatively low compared to those of most other snake species, and were extremely variable among individuals. There were individuals with near-zero levels in most months. Overall, the reproductive biology of males on Guam is aseasonal. In light of this finding, the claim of seasonality in the putative source population is reassessed. Testosterone levels were independent of coelomic fat body mass, which was extremely variable among males and, in many cases, quite low. Observations on morphological features suggest that individual reproductive capacity increases disproportionately with increasing body size. The possibility of facultative aseasonal/seasonal reproduction in the species is discussed. The observed capacity for continuous spermatogenesis and its relative independence from body condition may facilitate the invasive capabilities of this ecologically damaging species.

Over the last century, many species of reptiles have been advertly or inadvertently introduced outside of their native ranges (Smith and Kohler, 1977; Wilson and Porras, 1983; Meshaka et al., 2004; Engeman et al., 2008), but few are known to have had major ecological or economic impacts. The Brown Treesnake (*Boiga irregularis*) is arguably the most notable exception to this phenomenon—it is the only reptile known to have caused the extinction of another vertebrate species (Savidge, 1987). Since its accidental arrival on Guam as a stowaway in the 1950s, the Brown Treesnake has been implicated in the extirpation of nine of Guam's 13 native species of forest birds (Savidge, 1987), an additional avian species (Mariana Crow, *Corvus kubaryi*) very recently (D. Vice, pers. comm., 2009), and two of Guam's native lizard species (Rodda and Fritz, 1992). At present, the Brown Treesnake continues to occur islandwide and at relatively high densities (Vice and Vice,

2004), apparently through subsistence on various other nonnative vertebrate species that remain numerous (Rodda et al., 1999a). Ongoing control efforts in and around Guam's ports and airfields continue to net thousands of snakes a year (Vice and Pitzler, 2002; D. Vice, pers. comm., 2009). Without appropriate sustained levels of intervention and containment (Vice and Vice, 2004), inadvertent transport of the snake off island is inevitable, and further establishment elsewhere is possible.

The seriousness of this threat has resulted in a considerable body of research on the snake's biology, ecology, and methods for control. Given that reproduction is a key element in the persistence of any population, it is surprising that information on the reproductive biology of the Guam population has become available only recently. Savidge (1987), who first documented the link between the snake and declining numbers of forest birds, conducted the first reproductive studies on Guam in the late 1980s while colonization was still in progress (Savidge et al., 2007). Follow-up

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studies conducted since full colonization have been more limited, with data collected for either only a few weeks of the year (Mathies et al., 2001; Aldridge and Arackal, 2005) or no data provided for morphological features (Moore et al., 2005). Using large samples of snakes collected in all months of the year, Savidge et al. (2007) found that the ductus deferentia in nearly all males examined were convoluted and thickened, strongly suggesting sperm were stored throughout the year. That there were no observable changes in testis mass throughout the year further suggested testes were continuously spermatogenic. But it remains equivocal whether spermatogenesis in Guam males is seasonal, as has been observed in the majority of tropical snake species, or aseasonal. Aseasonal spermatogenesis has been adequately documented in only a few species of tropical snakes and only at the population level (Berry and Lim, 1967) not at the level of the individual (Licht, 1984). A possible exception is the marine snake *Laticauda colubrina*; only one of 160 adult males examined (sample size no less than six in any month) had a regressed germinal epithelium (Gorman et al., 1981). The other major aspect of the squamate urogenital system that requires investigation to fully assess male reproductive state, and one that has recently received some attention in male Brown Treesnakes, is steroidogenic activity. Based on examination of levels of plasma testosterone in a sample of adult male Brown Treesnakes collected in July on Guam, Mathies et al. (2001) concluded that only one of 35 males was reproductive. However, levels of testosterone peripheral to the testes are not necessarily indicative of spermatogenic activity (Licht, 1984). In a much larger study than that conducted by Mathies et al. (2001), with samples from nearly all months, Moore et al. (2005) found what they interpreted as basal levels of plasma testosterone in all months, with only June levels being significantly higher than that of any other month. Because they also found elevated plasma levels of the stress hormone corticosterone, they concluded that the low levels of testosterone observed were atypical and that the Guam population was in decline. However, no data on condition of the testis or accessory ducts were presented.

The objective of this study was to use monthly examinations of the urogenital system organs and plasma testosterone levels of male *B. irregularis* to provide a relatively complete understanding of male reproductive cycle on Guam. Such information could provide insights into the capacity of this species to invade other nonindigenous areas and direct development of control methods such as an orally delivered reproductive inhibitor (T. Mathies, Reproductive Inhibition as a Tool for Controlling the Brown Treesnake on Guam, 2008, unpubl. data) or a sex pheromone attractant (Mason, 1999). From a larger perspective, this is one of the few studies to provide a comprehensive investigation of the reproductive biology of a tropical snake using samples collected from the same area in every month of the year.

MATERIALS AND METHODS

Animal and Sample Collection.—Guam is located in the western Pacific, 13° north of the Equator, and has a dry season from January to May and a rainy season from July to November. Monthly mean air temperatures range from about 27°C in January to about 28°C in June. Male Brown Treesnakes were collected in northern Guam in every month of the year from 3 May 2001 to 19 April 2002. Snakes were collected at night when they are active by driving alongside cyclone fence lines using a spotlight to sight snakes crawling on the fences. Upon sighting a snake, a blood sample was obtained as quickly as possible by restraining the snake on the fence, clipping off part of the tail (i.e., for individuals posthumously verified as male), and then collecting blood into heparinized lithium vials. Blood samples were collected in less than 8 min of when a snake was first sighted from the vehicle. Snakes were euthanized immediately afterward by placing them in a cloth bag on a wire mesh stand on a cloth pad soaked with Halothane® (Ayerst Laboratories, New York City) within a sealed plastic container. Blood samples and carcasses were then placed in a cooler on ice. Carcasses were placed in 10% neutral buffered formalin; blood samples were centrifuged, and plasma was stored at −20°C within 1–8 h of when they were obtained. Blood plasma was transported frozen to the Center for Reproduction of Endangered Species in San Diego, California, and stored at −20°C until analysis.

Morphological Measurements.—Snout–vent length (SVL) was measured to the nearest 1 mm. Testes, kidneys, and coelomic fat bodies (CFM) were dissected out and weighed to the nearest 0.01 g. Histological sections for testes, the efferent ducts, and kidneys were prepared using standard histological methods and were stained with hematoxylin and eosin. Slides were examined and photographed on a Leica DMLS microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany) with a MicroPublisher 5.0 RTV Non-Cooled camera (QImaging, Surrey, British Columbia, Canada) interfaced with Photoshop 6.0 (Adobe Systems, Inc., San Jose, CA). Measurements were made using an ocular

micrometer. Ten measurements per slide were made for seminiferous tubule width (STW), seminiferous tubule epithelial height (STEH), epididymidis sperm column cross-sectional length (ESL), epididymidis epithelial height (EEH), kidney sexual segment (KSS) tubule diameter, and KSS epithelial height. The ESL was measured as follows. Because the epididymidis was highly convoluted and was always hit multiple times in the same cross-section, measurements were made on the section of the epididymidis containing the largest sperm mass. Means for each of the above features were computed for each individual, and individual means were used in the analyses. Cross-sections of the ductus deferens adjacent to the kidney were examined, and the presence or absence of sperm was recorded. The criterion for sexual maturity was spermatozoa in the seminiferous tubules, ductus epididymis, or ductus deferens. Based on these parameters, 86 males were judged mature and included in this study.

Analyses of Plasma Testosterone (T).—Duplicate 100-μl aliquots of plasma were extracted in 2 ml of an ethyl acetate : hexane mixture (3 : 2) (in 75 × 100 ml glass tubes) by shaking for 30 sec on a vortex mixer (Lance and Elsey, 1986). Aqueous and organic phases were allowed to separate, and then the tubes were snap-frozen in a dry-ice-methanol mixture to freeze the aqueous layer. The organic phase was decanted into a 12 × 75 mm disposal glass tube and evaporated to dryness under a stream of filtered air in a 37°C water bath. The dried extract was dissolved in 500 μl of phosphate-buffered saline (PBS w/0.1% gel; pH 7.0), and the tubes were shaken on the vortex for 30 sec. Tritiated T (10,000 cpm) in 100 μl of PBS and 100 μl of antibody were added, and the mixture was allowed to equilibrate overnight at 4°C. Separation of bound from free steroid was achieved by addition of 250 μl of a charcoal-dextran-PBS mixture (1,000 ml PBS buffer, 0.625 g dextran and 6.25 g Norit A charcoal) per tube and allowing tubes to sit for 30 min at 4°C. All tubes were then centrifuged for 15 min at 8°C at 3,000 rpm ($\sim$ 1,400 × g). Following centrifugation, the supernatant containing the bound fraction was decanted into glass scintillation vials. Five milliliters of scintillation fluid (Packard Ultima Gold, PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA) was added, and the radioactivity was measured in a Beckman liquid scintillation spectrometer, LS 6500 (2 min per tube). Tritiated T was obtained from Du Pont NEN Products (Boston, MA). Antibody specific for T was purchased from ICN (Costa Mesa, CA). The assay was validated by parallelism of serially diluted samples of steroid-free Brown Treesnake plasma with the appropriate

standard curve. Assay sensitivity was 27 pg/ml, and the intraassay coefficient of variation was 4.3%.

Data Analyses.—Differences in reproductive parameters among months were evaluated using one-factor ANCOVA with SVL as the covariate. A homogeneity of slopes test was conducted for each analysis; interaction terms, when nonsignificant, were dropped from the model and the model recalculated. Differences among least-squares (LS) means were evaluated using the pairwise *t*-test option (PDIFF of PROC GLM) and are given ± 1 SE. These analyses were conducted using SAS® statistical software (vers. 9.1, 2002–2003, SAS Institute, Inc., Cary, NC). Because of the large number of ANCOVAs conducted (10), *P*-levels for these analyses were Bonferroni-adjusted with statistical significance set at $P < 0.005$.

Allometries of potential correlates of male reproductive capacity (e.g., T levels) were investigated by pooling data from all months. Pooling was reasonable given the lack of significant differences in most features among months (see Results). Lean body mass (total body mass – CFM), CFM, testis mass, STEH, ESL, plasma T, and KSS epithelial height were regressed on SVL using reduced major axis regressions (Rayner, 1985). These data were log-transformed prior to analysis. Analyses were conducted using the statistical package PAST (Hammer et al., 2001).

Finally, we investigated whether our data could explain variation in T levels and KSS condition. The CFM was a primary independent variable of interest because recent studies have suggested that Brown Treesnake reproduction on Guam has been depressed because of a shortage of prey (Jordan and Rodda, 1994; Moore et al., 2005). Backward stepwise regression was used to examine the relationship between plasma T and the independent variables testis mass and CFM adjusted for body size. Adjusted CFM was calculated as the residuals of the linear regression of CFM on SVL. The relationship between KSS tubule diameter and the independent variables T and adjusted CFM were examined similarly. T was included in this analysis because of its known influence on KSS activity (Bishop, 1959; Krohmer, 2004; Krohmer et al., 2004). These analyses were conducted using StatView® statistical software (vers. 5.0.1, 1999, SAS Institute, Inc., Cary, NC).

RESULTS

Seasonality of Reproduction.—All 86 males in this study had testes that were spermatogenic and showing no signs of either recrudescence or
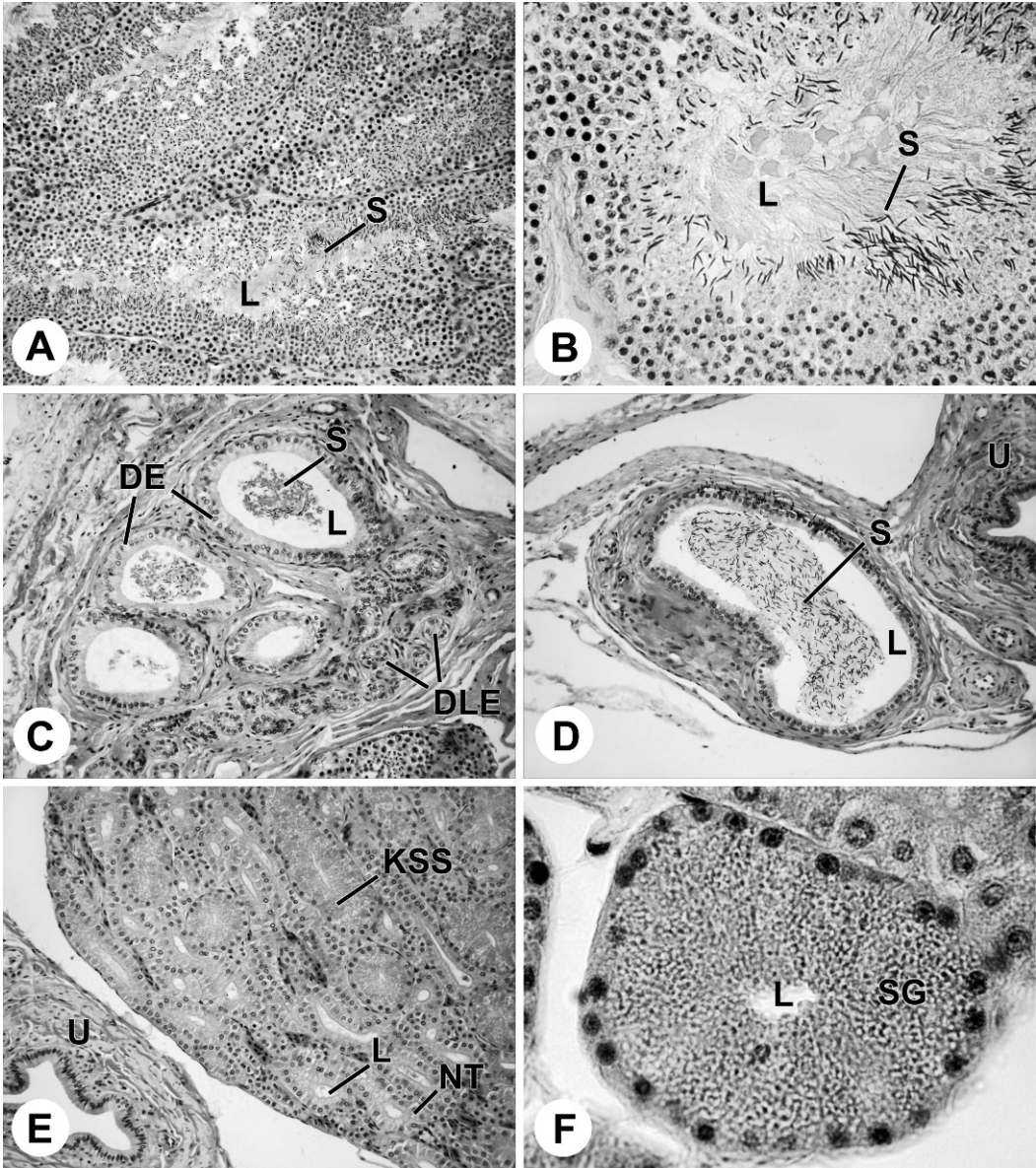
FIG. 1. Photomicrographs of cross-sections of testis seminiferous tubules, ductus epididymis, ductus deferens, and kidney sexual segment in mature male Brown Treesnakes (*Boiga irregularis*). Photomicrographs are representative of the 86 males included in the study. (A) Seminiferous tubules ($\times$ 20) showing spermatogenesis and sperm within tubule lumens; (B) single seminiferous tubule ($\times$ 40) showing all stages of spermatogenesis; (C) epididymis ($\times$ 20) with highly convoluted ductus epididymidis containing sperm; (D) ductus deferens ($\times$ 20) containing sperm; (E) kidney ($\times$ 20) showing representative density of sexual segments of tubules; (F) single kidney tubule ($\times$ 100) showing sexual segment with columnar cells (cell walls only partially discernable) filled with secretory granules. DE = ductus epididymidis; DLE = ductuli epididymidis; KSS = sexual segment of kidney tubule; L = lumen; NT = nephronic portion of kidney tubule; S = spermatozoa; SG = secretory granules; U = ureter.

regression (Fig. 1A, B). All males had sperm in the ductus epididymidis (Fig. 1C) and ductus deferens (Fig. 1D). Although we did not attempt to assess abundance of sperm in the ductus

deferens, there were no individual cases where sperm were sparse; cross-section area of the sperm column in the ductus deferens shown in Figure 1D is representative of males in all
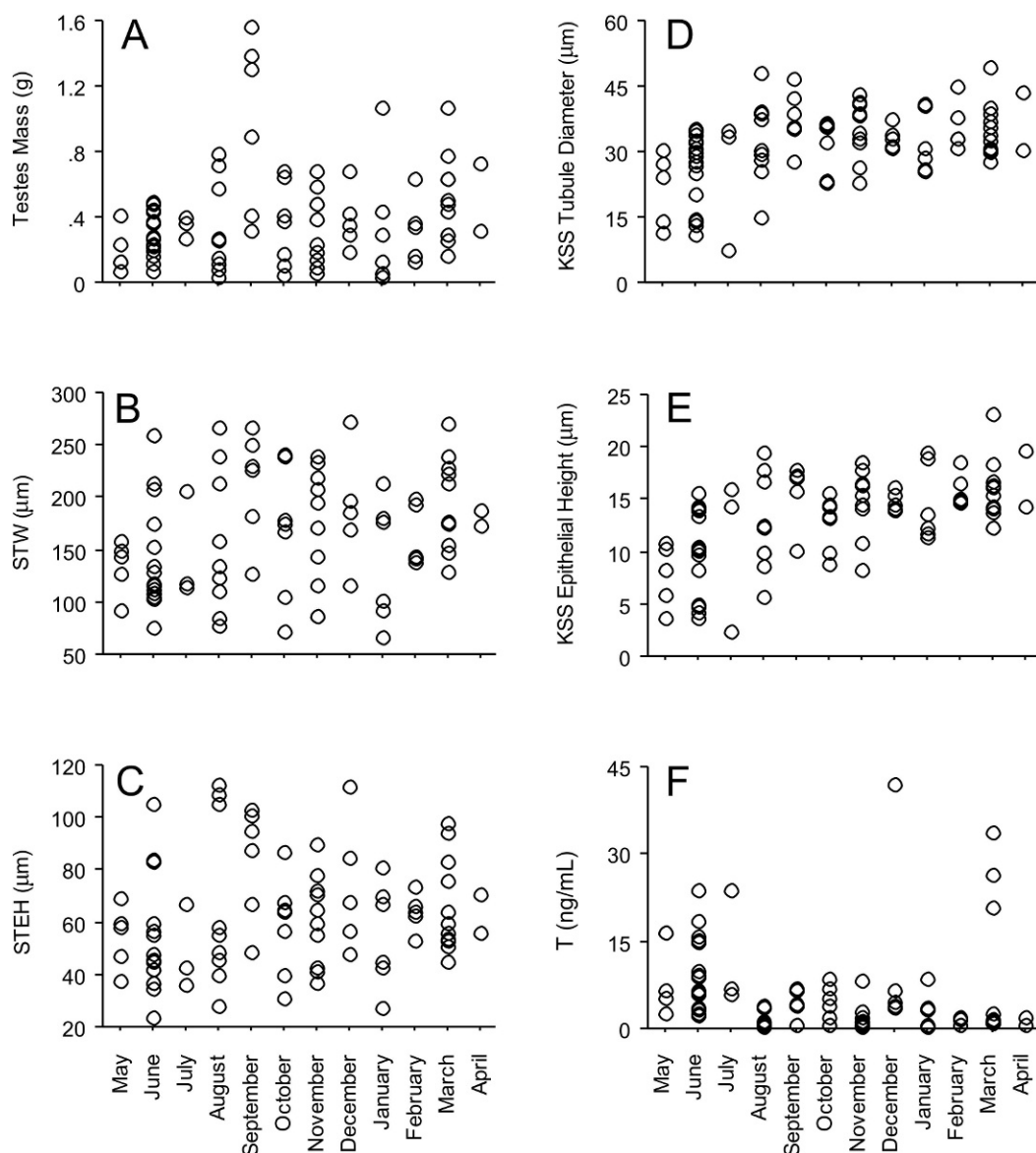
FIG. 2. Monthly variation in (A) testes mass, (B) seminiferous tubule width (STW), (C) seminiferous tubule epithelial height (STEH), (D) kidney sexual segment (KSS) tubule diameter, (E) KSS epithelial height, and (F) plasma testosterone (T) levels in mature male Brown Treesnakes (*Boiga irregularis*) on Guam.

months. In all males, kidneys were rather sparsely populated by KSS tubules (Fig. 1E). However, such tubules were always hypertrophied (Fig. 1E, F) and contained large eosinophile secretion granules in both the columnar cells and tubule lumens (Fig. 1F).

Variation in reproductive features among individuals by month is shown in Figure 2. Results for ANCOVAs are provided in Table 1, and LS means are provided in Table 2. Only mean KSS epithelial height varied significantly among months, with the lowest mean heights

observed May through July. The highest mean levels of T were observed May through July and December, but differences among months were not significant. The highest values of T were observed in one individual collected in December and another individual collected in March. In general, T was extremely variable among individuals and there were individuals with near-zero levels in most months. More than half of the males (63%) had T levels of 5 ng/ml or less (Fig. 3). Mean T was 6.16 ± 7.86 ng/ml; the median was 3.54 ng/ml.

TABLE 1. Statistical tests (analysis of covariance) for the effects of month on testis mass, seminiferous tubule width, seminiferous tubule epithelial height, epididymidis sperm mass length, epididymidis epithelial height, plasma testosterone, combined kidney mass, kidney sexual segment (KSS) tubule diameter, KSS epithelial height, and coelomic fat body mass in mature male Brown Treesnakes (*Boiga irregularis*) on Guam. Statistics for main effect and covariate are from models where nonsignificant ($P \geq 0.05$) interaction terms were dropped.

	Main effect: month	Covariate: snout–vent length	Interaction: month × snout–vent length
Combined testis mass (g)	$F_{1,85} = 1.59, P = 0.12$	$F_{1,85} = 264.72, P < 0.0001$	$F_{1,85} = 0.71, P = 0.73$
Seminiferous tubule width (µm)	$F_{1,85} = 1.52, P = 0.14$	$F_{1,85} = 61.11, P < 0.0001$	$F_{1,85} = 1.84, P = 0.07$
Seminiferous tubule epithelial height (µm)	$F_{1,85} = 1.34, P = 0.22$	$F_{1,85} = 56.51, P < 0.0001$	$F_{1,85} = 1.72, P = 0.09$
Epididymidis sperm mass length (µm)	$F_{1,78} = 0.90, P = 0.55$	$F_{1,78} = 44.61, P < 0.0001$	$F_{1,78} = 1.69, P = 0.10$
Epididymidis epithelial height (µm)	$F_{1,80} = 1.93, P = 0.05$	$F_{1,80} = 10.27, P = 0.002$	$F_{1,80} = 1.99, P = 0.05$
Combined kidney mass (g)	$F_{1,82} = 0.71, P = 0.72$	$F_{1,82} = 209.94, P < 0.0001$	$F_{1,82} = 1.30, P = 0.23$
KSS tubule diameter (µm)	$F_{1,85} = 2.62, P = 0.007$	$F_{1,85} = 11.22, P = 0.001$	$F_{1,85} = 0.98, P = 0.47$
KSS epithelial height (µm)	$F_{1,85} = 3.57, P = 0.0005$	$F_{1,85} = 10.21, P = 0.002$	$F_{1,85} = 1.10, P = 0.38$
Coelomic fat body mass (g)	$F_{1,85} = 0.86, P = 0.58$	$F_{1,85} = 30.05, P < 0.0001$	$F_{1,85} = 1.58, P = 0.13$
Plasma testosterone (ng/ml)	$F_{1,82} = 2.57, P = 0.008$	$F_{1,82} = 5.90, P = 0.02$	$F_{1,82} = 1.22, P = 0.30$

Allometric Relationships.—The smallest and largest adult males in our sample were 886 mm and 1,720 mm SVL, respectively. Relationships of morphological and physiological (T) features and body size are shown in Figure 4. The reduced major axis regression slopes for lean body mass, CFM, testis mass, STEH, ESL, plasma T, and KSS epithelial height, were significantly different from isometry and allometries were positive (Table 3). Thus, these features increased disproportionately with increasing body size. However, the CFM was extremely variable with respect to SVL accounting for only 40% of the observed variation. Fifteen percent of males had a CFM of 5 g or less. Although larger males had disproportionately higher levels of T than smaller males, SVL accounted for only 33% of the observed variation in T.

Predictors of T and KSS Condition.—Plasma levels of T were not related to either testis mass or the residuals of the regression of CFM on SVL (Backward Stepwise Regression: $R^2_{2,83} = 2.3; P = 0.13$). Likewise, KSS tubule diameter was not related to either T or the CFM residuals (Backward Stepwise Regression: $R^2_{2,82} = 0.65; P = 0.42$).

DISCUSSION

Seasonality at Population and Individual Levels.—We found no evidence for seasonality of spermatogenesis in male Brown Treesnakes. All individuals in this study had testes that were spermatogenic, and epithelial development within the seminiferous tubules was comparable to maximal extents observed within tubules of temperate (see Krohmer and Al-

dridge, 1985; Kuchling, 1991; Clesson et al., 2002) and subtropical snake species (see Lofts et al., 1966; Tsai and Tu, 2000). There was no significant variation in testis mass, STW or STEH among months, and the ductus deferens in every male was filled with sperm. Consequently, sperm production in males is unequivocally continuous with no evidence for variation in rate of production among months. However, the KSS epithelial height did show significant variation among months, with lower mean heights observed May through July. This pattern was paralleled by KSS tubule diameter, although differences among months were not significant. Secretory granules were present within the KSS columnar cells and tubule lumens of all males. However, the apparent seasonality, but incomplete regression of the KSS, is not unusual; in many snake species, the KSS shows only a brief period of partial regression (Fox, 1977; Saint Girons, 1982; Aldridge et al., 1995), and KSS tubules are often secretory over most of the year, even in some species where reproduction is strongly seasonal (Krohmer and Aldridge, 1985; Aldridge, 2002). However in other species, regression of the KSS can be substantial, reverting entirely to the mucous state (Saint Girons and Pfeffer, 1971; Saint Girons, 1982; Kuchling, 1991). Although the reduction in KSS epithelial height we observed in our May through July samples may represent decreased rates of KSS granule production, it is unlikely that variation in this feature imposes seasonality on Guam males, at least in terms of their capacity to effectively fertilize females.

Although we did not quantify KSS tubule density in kidney cross-sections, relatively few

TABLE 2. Summary of data for each month of the year on testis mass, seminiferous tubule width (STW), seminiferous tubule epithelial height (STEH), epididymidis sperm mass length (ESM), epididymidis epithelial height (EEH), plasma testosterone (T), combined kidney mass, kidney sexual segment (KSS) diameter, KSS epithelial height, and coelomic fat body mass (CFM) for mature male Brown Treesnakes (*Boiga irregularis*) on Guam. Sample sizes are given in parentheses, followed by least-square means ± 1 SE. Months with significantly different means (KSS epithelial height only) are designated with different letters.

|	Testes			Ductus epididymis				KSS		
	Mass (g)	STW (µm)	STEH (µm)	ESM (µm)	EEH (µm)	T (ng/ml)	Kidney mass (g)	Diameter (µm)	Epithelial height (µm)	CFM (g)
May	(5) 0.38 ± 0.11	(5) 164 ± 18	(5) 65.5 ± 7.2	(5) 10.9 ± 2.3	(5) 4.1 ± 0.5	(5) 11.15 ± 3.27	(5) 1.32 ± 0.15	(5) 23.8 ± 3.3	(5) 8.8 ± 1.6 a	(5) 16.19 ± 4.96
June	(17) 0.35 ± 0.03	(17) 155 ± 10	(17) 55.8 ± 3.8	(16) 7.1 ± 1.3	(16) 3.1 ± 0.3	(17) 10.24 ± 1.75	(17) 1.34 ± 0.08	(17) 25.8 ± 1.8	(17) 10.4 ± 0.8 a	(17) 16.21 ± 2.65
July	(3) 0.52 ± 0.25	(3) 167 ± 23	(3) 56.3 ± 9.1	(3) 6.0 ± 3.6	(3) 3.1 ± 0.6	(3) 13.48 ± 4.15	(3) 1.34 ± 0.19	(3) 26.7 ± 4.2	(3) 11.6 ± 2.0 ab	(3) 13.75 ± 6.31
August	(9) 0.36 ± 0.04	(9) 160 ± 13	(9) 68.4 ± 5.2	(8) 6.3 ± 1.8	(8) 3.2 ± 0.5	(6) 1.82 ± 2.91	(9) 1.51 ± 0.11	(9) 32.8 ± 2.4	(9) 12.7 ± 1.1 ab	(9) 18.38 ± 3.63
September	(6) 0.47 ± 0.08	(6) 129 ± 20	(6) 52.2 ± 7.7	(6) 3.8 ± 2.3	(6) 3.2 ± 0.5	(6) 0 ± 3.51	(6) 1.34 ± 0.19	(6) 31.4 ± 3.5	(6) 13.0 ± 1.7 ab	(6) 19.08 ± 5.31
October	(7) 0.31 ± 0.05	(7) 162 ± 15	(7) 56.4 ± 5.9	(7) 5.7 ± 1.9	(7) 4.0 ± 0.4	(7) 3.63 ± 2.70	(7) 1.17 ± 0.12	(7) 31.2 ± 2.7	(7) 12.6 ± 1.3 ab	(7) 7.68 ± 4.11
November	(10) 0.38 ± 0.04	(10) 179 ± 13	(10) 64.5 ± 5.0	(9) 8.0 ± 1.7	(9) 3.2 ± 0.3	(10) 2.51 ± 2.27	(10) 1.35 ± 0.10	(10) 36.0 ± 2.3	(10) 15.4 ± 1.1 ab	(10) 13.09 ± 3.45
December	(5) 0.43 ± 0.06	(5) 196 ± 18	(5) 76.9 ± 7.0	(4) 11.6 ± 2.6	(4) 4.7 ± 0.5	(5) 12.71 ± 3.20	(5) 1.18 ± 0.14	(5) 34.0 ± 3.2	(5) 15.1 ± 1.5 ab	(5) 8.52 ± 4.87
January	(6) 0.33 ± 0.0	(6) 138 ± 16	(6) 55.2 ± 6.4	(6) 7.9 ± 2.1	(6) 3.5 ± 0.4	(5) 2.86 ± 2.91	(5) 1.42 ± 0.13	(5) 32.1 ± 3.0	(6) 14.6 ± 1.4 ab	(6) 14.62 ± 4.44
February	(5) 0.44 ± 0.07	(5) 176 ± 18	(5) 68.9 ± 7.0	(5) 5.4 ± 2.3	(5) 4.4 ± 0.4	(5) 2.14 ± 3.21	(5) 1.28 ± 0.14	(5) 37.1 ± 3.2	(5) 16.4 ± 1.5 bc	(5) 13.40 ± 4.88
March	(11) 0.49 ± 0.04	(11) 192 ± 12	(11) 66.0 ± 4.7	(9) 8.6 ± 1.7	(10) 3.8 ± 0.3	(11) 8.32 ± 2.15	(11) 1.43 ± 0.10	(11) 35.0 ± 2.2	(11) 15.7 ± 1.0 bc	(11) 19.36 ± 3.28
April	(2) 0.49 ± 0.09	(2) 174 ± 29	(2) 61.1 ± 11.1	(2) 5.4 ± 3.4	(2) 4.5 ± 0.7	(2) 0.96 ± 5.05	(2) 1.45 ± 0.23	(2) 36.4 ± 5.1	(2) 16.7 ± 2.4 ab	(2) 9.74 ± 7.68
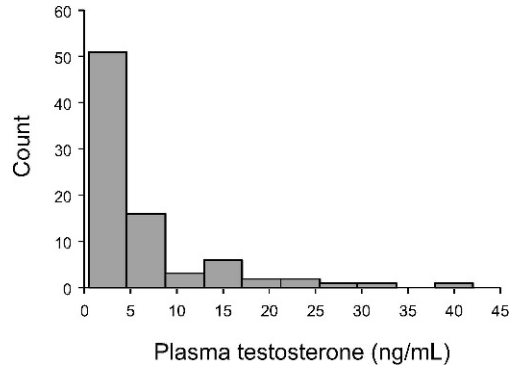
FIG. 3. Frequency histogram of plasma testosterone levels in mature male Brown Treesnakes (*Boiga irregularis*) on Guam. Data are for males captured in all months of the year.

KSS were present compared to that seen in snake species where testes undergo seasonal regression (see Volsøe, 1944; Saint Girons and Pfeffer, 1971; Kuchling, 1991; Aldridge, 2002). Perhaps the relatively low KSS densities we observed translate to a relatively low overall rate of granule production, this low rate being adequate for species where sperm production is not strongly seasonal and rapid. However, comparative data on other species with continuous or nearly continuous spermatogenesis are lacking, but methods for quantifying KSS tubule density are available (Krohmer, 2004; Krohmer et al., 2004).

The relationship between androgens and KSS activity in squamates has been investigated extensively, and it is well established that KSS hypertrophy occurs in response to androgens (Bishop, 1959; Pandha and Thapliyal, 1964; Prasad and Reddy, 1972; Krohmer et al., 2004). However, in the present study the lowest means observed for KSS epithelial height were temporally associated with some of the highest mean levels of T. Activity of the KSS in male Brown Treesnakes certainly does not seem to be finely regulated by plasma T; as revealed by backward stepwise regression analysis, T did not significantly account for any variation in KSS tubule diameter.

Few studies have conducted direct assays of plasma androgen levels in snakes with continuous (or nearly so) reproduction. This is primarily the result of the relative scarcity of this reproductive pattern and, to a lesser extent, its apparent confinement to the lesser-studied tropical species (Licht, 1984). In all snake species studied to date (tropical and temperate) where there is a well-defined mating season, mating activity is temporally associated with elevated plasma levels of T and frequently peak annual levels (see Naulleau et al., 1987; Bonnet and
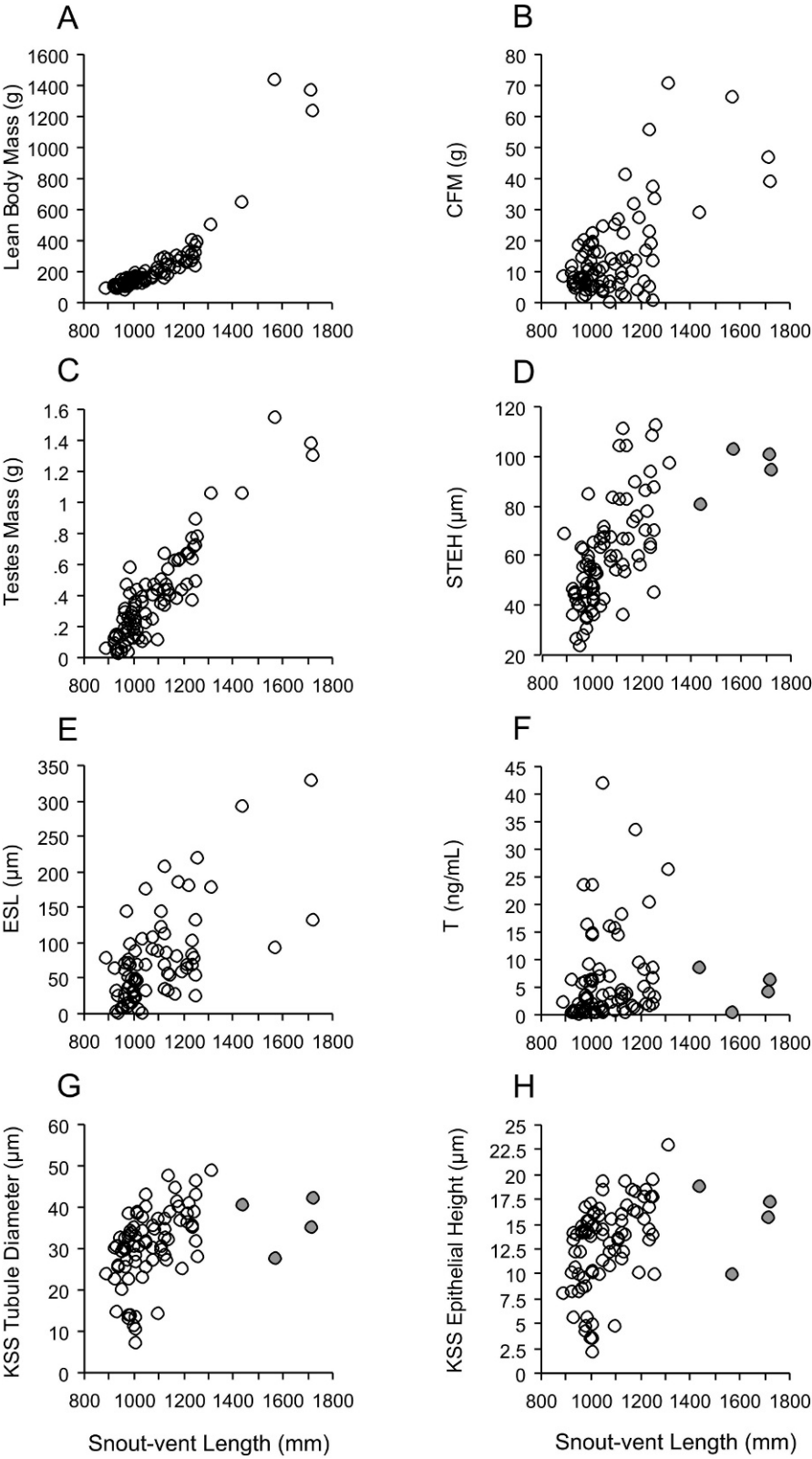
TABLE 3. Scaling relationships in mature male Brown Treesnakes (*Boiga irregularis*) evaluated using reduced major axis regressions. The independent variable in all analyses was snout–vent length. Data were log-transformed prior to analysis. For analyses of seminiferous tubule epithelial height, plasma testosterone, and kidney sexual segment (KSS) epithelial height, the four largest snakes were deemed outliers and excluded from the regressions (see Fig. 4).

Dependent variable	Slope	95% CI	r	P	N
Lean body mass	4.15	3.84–4.46	0.96	< 0.001	86
Coelomic fat body mass	6.60	5.16–8.44	0.40	< 0.001	86
Testis mass	6.25	5.17–7.96	0.78	< 0.001	86
Seminiferous tubule epithelial height	3.47	2.93–4.06	0.65	< 0.001	82
Epididymidis sperm mass length	6.97	5.26–9.28	0.53	< 0.001	74
Plasma testosterone	12.69	10.73–15.07	0.33	< 0.001	79
KSS epithelial height	4.50	3.28–5.73	0.40	< 0.001	82

Naulleau, 1996; Zaidan et al., 2003; Taylor et al., 2004). In this study, mean levels of T did not vary significantly among months, and although the highest mean levels occurred in the May through June samples, the comparably high levels in the December and March samples negate any recognizable trend. These findings suggest that there is no well-defined mating season in the Guam population. Mating has not been observed on Guam (Rodda et al., 1999b), perhaps because they are nocturnal and cryptic.

The levels of plasma T we observed deserve comment in that they were low compared to other snake species. In seven of 12 months we sampled, mean T-levels were less than 5 ng/ml, which is similar to basal levels observed in many temperate zone species (see Krohmer et al., 1987; Bonnet and Naulleau, 1996; Moore et al., 2001; Zaidan et al., 2003). Further, the highest mean levels of T we observed were considerably less than peak monthly levels in the aforementioned studies (but see Weil and Aldridge, 1981). Thus, it is apparent that spermatogenesis in male Brown Treesnakes does not require even moderate levels of plasma T—individuals with near-zero levels of T exhibited normal spermatogenesis. We also observed considerable variation of T among individuals within months. Some sources of this variation can be addressed. First, we found that T levels increase with increasing body size. Second is the consideration that males have cyclic levels of T but are cycling asynchronously. Such activity would be obscured under our sampling method. However, if we assume males are asynchronously cycling between similar high and low levels of T, we would expect a fairly equal count distribution of T-levels in our sample. Inspection of Figure 3 shows that this is not the case; males with T-levels above ~ 10 ng/ml are underrepresented and become increasingly scarce toward the highest levels observed, whereas males with T levels less than ~ 10 ng/ml are overrepresented and become increasingly prevalent toward the lowest levels observed. Third, the influence of CFM on male reproduction has received little attention, but in at least one species (*Coluber viridiflavus*), observations suggest that most of the body reserves are spent on reproduction, primarily through actively searching for females (Bonnet and Naulleau, 1996). For male Brown Treesnakes, reproductive processes per se are not noticeably affected by fat reserves, either positively or negatively. Male Brown Treesnakes tend to have higher relative CFM with increasing body size, but variation among individuals is substantial. None of the variation in testis mass was attributable to CFM. Even males with very low CFM's were spermatogenic and had sperm in the ductus deferens. Given the observations for *C. viridiflavus*, it might be surmised that male Brown Treesnakes with low CFMs are the ones actively searching for females. If so, such males might be expected to have high levels of T. However, none of the observed variation in plasma T was attributable to CFM. Thus, the cause of the substantial variation in CFM is unknown, but it is clear that male Brown Treesnakes can be spermatogenic

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FIG. 4. Relationships between morphological and physiological features and body size in mature male *Boiga irregularis*. (A) Lean body mass; (B) coelomic fat body mass (CFM); (C) testes mass; (D) seminiferous tubule epithelial height (STEH); (E) ductus epididymidis sperm mass length (ESL); (F) plasma testosterone (T); (G) kidney sexual segment (KSS) tubule diameter; (H) KSS epithelial height. Reduced major axis regression slopes were statistically significant and indicative of positive allometry. In D, F, G, and H, the four largest snakes (filled circles) were considered outliers and excluded from the regressions. For reduced major axis regression statistics, see Table 3.

even when their fat body masses are close to minimal. Finally, it is possible that the high levels of T seen in some individuals were induced through recent encounters with reproductive females or fights with other males. Mating-induced increases in plasma T have been observed in some vertebrates (Lunstra et al., 1989; Villani et al., 2006), but similar studies have not been conducted on snakes. Schuett et al. (1996) found that staged fights between male copperheads did not elevate plasma T in winners nor depress T in losers. Why some male Brown Treesnakes exhibit high levels of T relative to their body size remains unknown.

Reproduction on Guam and in the Native Range.—Our findings for males in the Guam population contrast with those for males in parts of the species' native range. Bull et al. (1997) examined monthly collections of males from subtropical southeastern Queensland and equatorial Papua New Guinea (PNG) and associated islands and found that spermatogenesis in males from southeastern Queensland was strongly seasonal with complete testicular regression occurring during the warmer months. They also concluded that spermatogenesis in PNG males was seasonal but with a comparatively brief period of regression from January to March. However, Aldridge and Arackal (2005) presented a different assessment of the Bull et al. (1997) data for PNG males, positing that four of the five males comprising the January through March sample may have been immature or not fully mature. In our view, the assessment of Aldridge and Arackal (2005) that the contended period of testicular regression in the PNG population is not adequately supported seems justified. Because the Guam population of Brown Treesnakes is thought to have originated from the Admiralty Islands at 2°S, just north of PNG (Rodda et al., 1992), there may be no real disagreement between patterns of testicular activity we and others (Savidge et al., 2007) observed on Guam and those observed in the putative source population by Bull et al. (1997).

Male Body Size and Reproductive Capacity.—The range of body size among mature males is considerable with some individuals maturing at only about half their potential maximum body size. Large body size seems to be a particularly important feature of male Brown Treesnake reproductive biology. In contrast to most colubrid species, male Brown Treesnakes attain considerably larger body sizes than females (Savidge, 1991). Like other colubrids, male Brown Treesnakes engage each other in combat (Greene and Mason, 2000) whereby competing males intertwine and attempt to pin and constrict each other (Greene and Mason, 2000;

TM, unpubl. data). In contests staged in the laboratory, the winner was always the larger male (Greene and Mason, 2000), and for snakes in general, large male size seems to be an adaptation to intrasexual competition (Shine, 1978, 1994). The allometry of body size in male Brown Treesnakes is such that males become increasingly ''stocky'' as they increase in SVL, a condition that may confer greater ability in gaining successful matings. The disproportionate increase in body mass primarily is cause by the increase in lean body mass relative to SVL but is augmented by the parallel allometric relationship between CFM and SVL. Thus, as a male increases in SVL, and assuming it has a ''normal'' CFM, it gains an increasing greater body mass advantage over smaller males. Concomitant with increasing body mass is an increase in level of plasma T, although even when values for the three largest males were omitted, SVL accounted for only 33% of the observed variation. The relatively high T levels were observed in individuals in the 1,000–1,300 mm SVL range; thus males smaller than 1,000 mm may be at an added competitive disadvantage for matings.

Allometries of features of the testes and KSS in mature males indicate that these features continue development as males increase in body size. Testis mass increases faster than SVL with no apparent asymptote within the range of body sizes in our sample. The largest individual in our sample (1,720 mm SVL) was only 260 mm shorter than the largest male in a substantial sample ($N = 283$) of males collected in nonurban areas on Guam (Savidge, 1991), and males of these sizes are uncommon. Thus, male Brown Treesnakes exhibit indeterminate testes growth over most or all of the range of body sizes observed on Guam. Other features are more indicative of true maturation. Epithelial height of the seminiferous tubules increases faster than body size but appears to reach a maximum height at about 1,200 mm SVL. A similar pattern was observed for KSS tubule diameter and epithelial height. Thus, even though the testes and kidneys continue to increase in mass as a male matures, the diameters and epithelial heights of seminiferous and kidney tubules attain maximums. This further ''maturation'' appears to be complete by the time males reach about 1,300 mm.

There is no information on the rate of sperm production in male Brown Treesnakes. Assuming the rate does not vary with body size, larger males would have a greater sperm production than smaller males simply by virtue of their larger testes. In support, ESL in male Brown Treesnakes had a positive allometry with SVL. Because the epididymis is not known to be a

storage area for sperm in snakes, this is more compelling evidence for increasing volume of sperm production with larger body size. However, it is unknown whether large male Brown Treesnakes are better able to fertilize females strictly on this basis.

Potential for Invasiveness.—Assuming the eastern Australian form of the Brown Treesnake and forms from the more tropical areas of the species range constitute a single species (for discussion, see Rodda et al., 1999b; Whittier et al., 2000), then the observed population differences in male patterns of testicular activity may be genetically determined or, depending on the wide range of climatic conditions throughout its substantial latitudinal range, facultative. The ability of individuals to facultatively switch between asynchronous continuous reproduction (e.g., Guam population) to synchronized seasonal reproduction (e.g., southeastern Queensland population), if present, would greatly increase the invasive capacity of this species. There is direct evidence for this capacity in both sexes; after exposing males of Guam origin to a five-week period of ''cooling'' ending in mid-January (see Greene and Mason, 2000), males showed a synchronous and marked elevation of T in March and April, a seasonal type pattern that was interpreted as the species ''norm'' (Moore et al., 2005). This observation contrasts sharply with the relatively low and invariant levels of T we observed in free-ranging Guam males. In Guam, females with enlarged ovarian follicles have been found in most (McCoid, 1994; TM, unpubl. data) or all months of the year (Savidge et al., 2007) demonstrating females there have aseasonal reproduction. However, when Guam females are brought into captivity and placed under a temperature regime simulating the cool to warm transition of a seasonal climate, females initiated ovarian follicular growth in synchrony (Mathies and Miller, 2003, 2004; TM, unpubl. data). Thus, both sexes in the Guam population are apparently capable of alternating between aseasonal and seasonal type reproductive patterns in response to local environmental temperatures. Such plasticity has obvious implications for the potential geographical scope of invasion.

Overall, our findings suggest that the reproductive biology of male Brown Treesnakes enhances the species' invasive capabilities. Care should be taken to prevent transporting it to other vulnerable locations in the tropics, subtropics, and even more temperate climates.

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